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(21) International Application Number: PCT/US00/05537 (22) International Filing Date: 2 March 2000 (02.03.00) (30) Priority Data: 60/124,118 12 March 1999 (12.03.99) US (71) Applicant (for all designated States except US): REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): ECONOMIDES, Aris, N. [GR/US]; 12 Mt. Morris Park West, New York, NY 10027 (US). (74) Agents: PALLADINO, Linda, O.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: NOVEL NUCLEIC ACIDS AND POLYPEPTIDES (57) Abstract <p>DAN/Cerberus Related protein 6 (DCR6) polypeptides and related nucleic acids are provided. Included are natural (DCR6) homologs from several species and polypeptides comprising a (DCR6) domain having specific activity. The polypeptides may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are isolated hybridization probes and oligonucleotide primers capable of specifically hybridizing with the disclosed genes, specific binding agents and methods of making and using the subject compositions.</p>		

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NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

This International Application claims priority of U.S. Provisional Application No. 60/124,118, filed March 12, 1999. All publications and patent applications
5 cited in this specification are hereby incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

INTRODUCTION

10

Field of the Invention

The field of this invention is polypeptides which regulate cell function and, in particular, antagonize bone morphogenic proteins and which are involved in the
15 development and maintenance of the vascular system.

Background

Natural regulators of cellular growth, differentiation and function have
20 provided important pharmaceuticals, clinical and laboratory tools, and targets for therapeutic intervention. A variety of such regulators have been shown to have profound effects on basic cellular differentiation and developmental pathways. For example, the recently cloned cerberus protein induces the formation of head structures in anterior endoderm of vertebrate embryos. Similarly, the noggin
25 protein induces head structures in vertebrate embryos, and can redirect mesodermal fates from ventral fates, such as blood and mesenchyme, to dorsal fates such as muscle and notochord and can redirect epidermal fates to anterior neural fates. The activities of chordin are similar to those of noggin, reflecting a common mechanism of action - namely antagonizing bone morphogenic
30 proteins (BMPs) and thereby preventing their function. BMPs have diverse biological activities in different biological contexts, including the induction of

cartilage, bone and connective tissue, and roles in kidney, tooth, gut, skin and hair development.

Different members of the TGF β superfamily can instruct cells to follow different
5 fates, for example TGF β induces neural crest to form smooth muscle, while
BMP2 induces the same cells to become neurons. In Xenopus experiments,
dissociated animal cap cells (prospective ectoderm) become epidermis in
response to BMP4 but become mesoderm in response to activin.

10 Since the sequence identity between activin and BMP4 is low, it is not surprising
that they induce different fates. It is more surprising that members of the BMP
subfamily, which are quite closely related in sequence, can induce distinct fates.
A striking example results from implantation of a matrix impregnated with a
BMP into muscle; when the effects are monitored histologically, BMP2, 4 and 7
15 induce endochondral bone formation, whereas a related molecule BMP12/GDF7
induces connective tissue similar to tendon. Similarly, BMP4 can induce cell
death in the hindbrain neural crest, while the related protein dorsalin does not.

Since different BMP family members can induce different fates, then BMP
20 antagonists that have specificity in blocking subsets of BMPs could change the
balance of BMPs that are presented to a cell, thus altering cell fate. In view of the
importance of relative BMP expression in human health and disease, regulators
of cellular function and BMP function in particular, such as noggin and cerberus,
provide valuable reagents with a host of clinical and biotechnological
25 applications.

The ability of ligands to bind cells and thereby elicit a phenotypic response such
as development, differentiation, growth, proliferation, survival and regeneration
in such cells is often mediated through transmembrane receptors. The
30 extracellular portion of each receptor is generally the most distinctive portion of
the molecule, as it provides the protein with its ligand-recognizing characteristic.

In the case of receptor tyrosine kinases (RTKs), binding of a ligand to the extracellular domain results in signal transduction via an intracellular tyrosine kinase catalytic domain which transmits a biological signal to intracellular target proteins. For example, a gene encoding an endothelial cell transmembrane tyrosine kinase, originally identified by RT-PCR as an unknown tyrosine kinase-homologous cDNA fragment from human leukemia cells, was described by Partanen, et al., Proc. Natl. Acad. Sci. USA, 87: 8913-8917 (1990). This gene and its encoded protein are called "tie" which is an abbreviation for "tyrosine kinase with Ig and EGF homology domains." Partanen, et al. Mol. Cell. Biol. 12: 1698-1707 (1992).

It has been reported that tie mRNA is present in all human fetal and mouse embryonic tissues. Upon inspection, tie message has been localized to the cardiac and vascular endothelial cells. tie mRNA has been localized to the endothelia of blood vessels and endocardium of 9.5 to 18.5 day old mouse embryos. Enhanced tie expression was shown during neovascularization associated with developing ovarian follicles and granulation tissue in skin wounds. Korhonen, et al. Blood 80: 2548-2555 (1992). Thus tie has been suggested to play a role in angiogenesis, which is important for developing treatments for solid tumors and several other angiogenesis-dependent diseases such as diabetic retinopathy, psoriasis, atherosclerosis and arthritis.

Two structurally related rat TIE receptor proteins have been reported to be encoded by distinct genes with related profiles of expression. One gene, termed tie-1, is the rat homolog of human tie. Maisonpierre, et al., Oncogene 8: 1631-1637 (1993). The other gene, tie-2, may be the rat homolog of the murine tek gene, which, like tie, has been reported to be expressed in the mouse exclusively in endothelial cells and their presumptive progenitors. Dumont, et al. Oncogene 8: 1293-1301 (1993). Both genes were found to be widely expressed in endothelial cells of embryonic and postnatal tissues. Significant levels of tie-2 transcripts were also present in other embryonic cell populations, including lens epithelium, heart epicardium and regions of mesenchyme. Maisonpierre, et al.,

Oncogene 8: 1631-1637 (1993). The predominant expression of the TIE receptor in vascular endothelia suggests that TIE plays a role in the development and maintenance of the vascular system. This could include roles in endothelial cell determination, proliferation, differentiation and cell migration and patterning
5 into vascular elements. In the mature vascular system, TIE could function in endothelial cell survival, maintenance and response to pathogenic influences.

An angiogenic factor, which was originally called TIE-2 ligand-1 (TL1) but is also referred to as angiopoietin-1 (Ang1), has been identified that signals through the
10 TIE-2 receptor and is essential for normal vascular development in the mouse. By homology screening, an Ang1 relative has been identified and called TIE-2 ligand-2 (TL2) or angiopoietin-2 (Ang2). Ang2 is a naturally occurring antagonist for Ang1 and the TIE2 receptor. For a description of the cloning and sequencing of TL1 (Ang1) and TL2 (Ang2) as well as for methods of making and uses thereof,
15 reference is hereby made to PCT International Publication No. WO 96/11269 published 18 April 1996 and PCT International Publication No. WO 96/31598 published 10 October 1996 both in the name of Regeneron Pharmaceuticals, Inc.; and S. Davis, et al., Cell 87: 1161-1169 (1996) each of which is hereby incorporated by reference.

20

The present invention relates to a novel regulator of cellular functions such as antagonizing bone morphogenic proteins and playing a role in the development and maintenance of the vascular system. This novel regulator shares homology with the DAN/cerberus family and is expressed in vascular tissues.

25

Relevant Literature

Bouwmeester, et al. (1996) Nature 382: 595-601 describe the cloning of Xenopus cerberus gene; Lamb, T. M., et al. (1993) Science 262: 713-718; Smith, W. C., et al.
30 (1992) Cell 70: 829-840; Smith, W. C., et al. (1993) Nature 361: 547-549; and Zimmerman, L. B., et al. (1996) Cell 86: 599-606 describe the isolation and function of the noggin protein. Piccolo, S., et al. (1996) Cell 86: 589-598; Sasai, Y., et al.

(1995) Nature 376: 333-336; and Sasai, Y., et al. (1994) Cell 79: 779-790 relate to the chordin protein. Enomoto et al. (1994) Oncogene 9: 2785-2791 and Ozaki, et al. (1996) Jpn. J. Cancer Res. 87: 58-61 describe human and murine homologs of the DAN gene.

5

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to DAN/Cerberus - Related protein 6 (DCR6) polypeptides and related nucleic acids. Included are
10 natural DCR6 homologs from different species, as well as polypeptides comprising a DCR6 domain and having DCR6-specific activity. The polypeptides may be produced recombinantly from transformed host cells with the subject nucleic acids. The invention provides isolated hybridization probes and primers capable of specifically hybridizing with the disclosed genes, specific binding agents
15 such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g., genetic hybridization screens for DCR6 transcripts), therapy (e.g., gene therapy to modulate DCR6 gene expression) and in the biopharmaceutical industry (e.g., reagents for screening chemical libraries for lead pharmacological agents).

20

Preferred applications of the subject DCR6 polypeptides include modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous DCR6 polypeptide under conditions whereby the added polypeptide specifically interacts with a
25 component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. Also preferred are methods for screening for biologically active agents, which methods involve incubating a DCR6 polypeptide in the presence of an extracellular DCR6 polypeptide-specific binding target and a candidate agent, under conditions whereby, but for the presence of
30 the agent, the polypeptide specifically binds the binding target at a reference affinity; detecting the binding affinity of the polypeptide to the binding target to determine an agent-biased affinity, wherein a difference between the agent-biased

affinity and the reference affinity indicates that the agent modulates the binding of the polypeptide to the binding target.

BRIEF DESCRIPTION OF THE FIGURES

5

Figure 1A-1F. The genomic DNA sequence of vts_hDCR6. The predicted boundaries of exons 1, 2, 3, and 4 are indicated underneath the sequence.

Figure 2A-2B. The nucleic acid and deduced amino acid sequence of vts_hDCR6 that was created by PCR-amplifying the individual exons from human genomic DNA and splicing them together. Silent mutations introduced to facilitate cloning and polypeptide expression are indicated in bold above the nucleic acid sequence and splice-junction sites between adjacent exons are underlined.

Figure 3A-3B. The nucleic acid and deduced amino acid sequence of hDCR6 that was cloned from a human kidney cDNA library having exons 1 and 4.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

An "oligonucleotide" or "oligonucleotide primer" or "primer" is a stretch of nucleotide residues which has a sufficient number of bases to be used in, for example, a polymerase chain reaction (PCR) or in DNA sequencing methodologies. These short sequences are based on (or designed from) genomic or cDNA sequences or back translated from protein sequences and are used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue or to initiate sequencing reactions. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

"Probes" are nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides, depending on use. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligonucleotides. They may be single- or double-stranded and carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

10

A "portion" or "fragment" of a polynucleotide or nucleic acid or polypeptide comprises all or any part of the polynucleotide or a polypeptide sequence having fewer nucleotides or amino acids than the complete polynucleotide or nucleic acid or polypeptide.

15

A "signal sequence" is a short amino acid sequence which can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

20

"Animal" as used herein may be defined to include human, domestic (i.e., cats, dogs), agricultural (i.e., cows, horses, sheep, goats, chicken, fish) or test species (i.e., frogs, mice, rats, rabbits, simians).

25 Since the list of technical and scientific terms cannot be all encompassing, any undefined terms shall be construed to have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

Furthermore, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to a

30 "restriction enzyme" or a "high fidelity enzyme" may include mixtures of such enzymes and any other enzymes fitting the stated criteria, or reference to the method includes reference to one or more methods for obtaining cDNA

sequences which will be known to those skilled in the art or will become known to them upon reading this specification.

Before the present sequences, variants, formulations and methods for making
5 and using the invention are described, it is to be understood that the invention is not to be limited only to the particular sequences, variants, formulations or methods described. The sequences, variants, formulations and methodologies may vary, and the terminology used herein is for the purpose of describing particular embodiments. The terminology and definitions are not intended to be
10 limiting since the scope of protection will ultimately depend upon the claims.

The invention provides DCR6 polypeptides which include natural DCR6 polypeptides and recombinant polypeptides comprising a DCR6 amino acid sequence, or a functional DCR6 polypeptide domain thereof having an assay-
15 discernable DCR6-specific activity. Accordingly, the polypeptides may be deletion mutants of the disclosed natural DCR6 polypeptides and may be provided as fusion products, e.g., with non-DCR6 polypeptides. The subject DCR6 polypeptide domains have DCR6-specific activity or function and are functionally distinct from each other and from DAN/Cerberus family and
20 noggin homologs. Such domains include at least 6 and preferably at least 8 consecutive amino acid residues of a natural DCR6 polypeptide (see human DCR6 sequence disclosed herein). Preferred DCR6 polypeptides comprise a DCR6 sequence conserved across species.

25 Note that contrary to prior art teachings which state that DAN is an intracellular zinc finger protein, applicants disclose that the natural DAN protein is extracellularly active as an antagonist of certain morphogenic proteins such as BMPs. In addition, the DCR5 sequence, set forth in co-pending US Provisional Application No. 60/097,296, filed August 20, 1998, is also extracellularly active as
30 an antagonist of certain morphogenic proteins such as BMPs. Because DCR-6 is structurally similar to DAN and DCR5, applicants predict that DCR6 will exhibit biological activities similar to these two related proteins. DCR6-specific activity

- or function may be determined by convenient in vitro, cell-based, or in vivo assays - e.g., in vitro binding assays, cell culture assays, in animals (e.g., immune response, gene therapy, transgenics). Binding assays encompass any assay where the specific molecular interaction of a DCR6 polypeptide with a binding target is evaluated. The binding target may be a natural binding target, a chaperon, or other regulator that directly modulates DCR6 activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a DCR6-specific agent such as those identified in assays described below. Generally, binding specificity is assayed by bioassay (e.g., the ability to induce neuronal tissue from injected embryonic ectoderm), target protein binding equilibrium constants (usually at least about 10^7 M⁻¹, preferably at least about 10^8 M⁻¹, more preferably at least about 10^9 M⁻¹), by the ability of the subject polypeptide to function as negative mutants in DCR6-expressing cells, by the ability to elicit DCR6-specific antibody production in a heterologous host (e.g., a rodent or rabbit).
- The claimed polypeptides may be isolated or pure - an "isolated" polypeptide is one that is no longer accompanied by some of the material with which it is associated in its natural state, and that preferably constitutes at least about 0.5%, and more preferably at least about 5% by weight of the total protein in a given sample; a "pure" polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total protein in a given sample. The subject polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).
- The subject polypeptides find a wide variety of uses including use as immunogens, targets in screening assays, bioactive reagents for modulating cell

growth, differentiation and/or function. For example, the invention provides methods for modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous DCR6 polypeptide under conditions whereby the added polypeptide specifically
5 interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. According to these methods, the extracellular surface includes plasma membrane-associated receptors; the exogenous DCR6 refers to a polypeptide not made by the cell or, if so, expressed at non-natural levels, times or physiologic locales; and suitable media include in
10 vitro culture media and physiological fluids such as blood, synovial fluid or lymph. Effective administrations of subject polypeptides may be useful in reducing undesirable (e.g., ectopic) bone formation, inhibit the growth of cells that require a morphogenic protein (e.g., BMP-dependent neuroblastomas and gliomas), alter morphogen-dependent cell fate/differentiation in culture, such as
15 with cells for transplantation or infusion. The polypeptides may be may be introduced, expressed, or repressed in specific populations of cells by any convenient way such as microinjection, promoter-specific expression of recombinant enzyme, or targeted delivery of lipid vesicles.

20 The invention provides natural and non-natural DCR6-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. DCR6-specific binding agents may include ligands such as BMPs, and receptors, such as somatically recombined protein receptors like specific antibodies or T-cell antigen receptors (See, e.g.,
25 Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and may also include other natural binding agents identified with assays such as one-, two- and three-hybrid screens, and non-natural binding agents identified in screens of chemical libraries such as described below. Agents of particular interest modulate DCR6 function.

30

The invention provides DCR6 nucleic acids, which find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR

primers, diagnostic nucleic acids, as well as use in detecting the presence of DCR6 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional DCR6 homologs and structural analogs.

- 5 The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e., no longer accompanied by some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural
10 sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Nucleic acids comprising the nucleotide sequence of Figure 2A-2B or Figure 3A-3B or fragments thereof, contain such sequences or fragments at a terminus, immediately flanked by a sequence other than that to which it is joined on a natural chromosome, or flanked by a native
15 flanking region fewer than 10 kb, preferably fewer than 2 kb, which is immediately flanked by a sequence other than that to which it is joined on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability.

20

- DCR6-encoding nucleic acids may be part of expression vectors and may be incorporated into recombinant host cells, e.g., for expression and screening, for transgenic animals, for functional studies such as the efficacy of candidate drugs for diseases associated with DCR6-mediated signal transduction. Expression
25 systems are selected and/or tailored to effect DCR6 polypeptide structural and functional variants through alternative post-translational processing.

- The invention also provides for nucleic acid hybridization probes and replication/amplification primers having a DCR6 cDNA specific sequence and
30 sufficient to effect specific hybridization with the sequences set forth in Figures 1A-1F, 2A-2B, or 3A-3B. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30%

- formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO₄, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to
- 5 washing at 42°C with 0.2x SSPE buffer at 42°C. DCR6 cDNA homologs can also be distinguished from other cDNA-encoding polypeptides using alignment algorithms, such as BLASTX (Altschul, et al. (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215: 403-410).
- 10 DCR6 hybridization probes find use in identifying wild-type and mutant alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. DCR6 nucleic acids are also used to modulate cellular expression or intracellular concentration or availability of active DCR6. DCR6 inhibitory nucleic acids are
- 15 typically antisense - single stranded sequences comprising complements of the disclosed natural DCR6 coding sequences. Antisense modulation of the expression of a given DCR6 polypeptide may employ antisense nucleic acids operably linked to gene regulatory sequences. Cells are transfected with a vector comprising a DCR6 sequence with a promoter sequence oriented such that
- 20 transcription of the gene yields an antisense transcript capable of binding to endogenous DCR6-encoding mRNA. Transcription of the antisense nucleic acid may be constitutive or inducible and the vector may provide for stable extrachromosomal maintenance or integration. Alternatively, single-stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given
- 25 DCR6 polypeptide may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results in a substantial reduction in expression of the targeted protein. An enhancement in DCR6 expression is effected by introducing into the targeted cell type DCR6 nucleic acids which increase the functional expression of the corresponding gene products. Such
- 30 nucleic acids may be DCR6 expression vectors, vectors which upregulate the functional expression of an endogenous allele, or replacement vectors for

targeted correction of mutant alleles. Techniques for introducing the nucleic acids into viable cells are known in the art and include retroviral-based transfection or viral coat protein-liposome mediated transfection.

- 5 The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of DCR6 modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate DCR6 interaction with a natural DCR6 binding target. A wide
10 variety of assays for binding agents are provided including protein-protein binding assays, immunoassays or cell based assays. Preferred methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds.

- In vitro binding assays employ a mixture of components including a DCR6
15 polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g., a tag for detection or anchoring. The assay mixtures comprise a natural DCR6 binding target. While native binding targets may be used, it is frequently preferred to use portions thereof as long as the portion provides binding affinity and avidity to the subject DCR6 that is conveniently measurable
20 in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds, preferably small organic compounds, and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents such as salts, buffers, neutral proteins,
25 e.g., albumin, detergents, protease inhibitors, nuclease inhibitors or antimicrobial agents, may also be included. The mixture components can be added in any order that provides for the requisite binding and incubations may be performed at any temperature which facilitates optimal binding. The mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological
30 agent, the DCR6 specifically binds the cellular binding target, portion or analog with a reference binding affinity. Incubation periods are chosen for optimal binding but are also minimized to facilitate rapid, high throughput screening.

After incubation, the agent-biased binding between the DCR6 and one or more binding targets is detected by any convenient way. For cell-free binding type assays, a separation step is often used to separate bound from unbound
5 components. Separation may be effected by, for example, precipitation or immobilization, followed by washing by, e.g., membrane filtration or gel chromatography. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection such as radioactivity, luminescence, optical or electron density, or indirect
10 detection such as an epitope tag or an enzyme. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g., through optical or electron density, radiative emissions, nonradiative energy transfers, or indirectly detected with antibody conjugates. A difference in the binding affinity of the DCR6 polypeptide to the target in the
15 absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the DCR6 polypeptide to the corresponding binding target. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

20

The invention provides for a method for modifying the physiology of a cell comprising an extracellular surface in contact with a medium, said method comprising the step of contacting said medium with an exogenous DCR6 polypeptide under conditions whereby said polypeptide specifically interacts with
25 at least one of a component of said medium and said extracellular surface to effect a change in the physiology of said cell.

The invention further provides for a method for screening for biologically active agents, said method comprising the steps of a) incubating a DCR6 polypeptide in
30 the presence of an extracellular DCR6 polypeptide specific binding target and a candidate agent, under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity; b)

detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

5

The invention provides for an isolated nucleic acid molecule encoding human DCR6.

10 The invention further provides for an isolated nucleic acid molecule having a sequence selected from the group consisting of (a) the nucleotide sequence comprising the coding region of human DCR6 as set forth in Figure 2A-2B; (b) the nucleotide sequence comprising the coding region of human DCR6 as set forth in Figure 3A-3B; (c) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) or (b) and which encodes a molecule
15 having the biological activity of human DCR6; or (d) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a), (b), or (c) and which encodes a molecule having the biological activity of the human DCR6.

20 The invention provides for a vector or plasmid wherein the DCR6 nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.

The invention further provides for isolated human DCR6 polypeptide
25 comprising the amino acid sequence as set forth in Figure 2A-2B or Figure 3A-3B, or a fragment thereof having DCR6-specific activity.

The invention provides for a host-vector system for the production of human DCR6 wherein the host cell is a bacterial, yeast, insect or mammalian cell.
30

The invention provides for a method of producing human DCR6 which comprises growing cells of a host-vector system under conditions permitting

production of the human DCR6, and recovering the human DCR6 so produced.

The invention also provides for an antibody which specifically binds the human DCR6 polypeptide. The antibody may be a polyclonal antibody or a monoclonal
5 antibody.

The invention provides for a pharmaceutical composition comprising human DCR6 polypeptide and an acceptable carrier as well as a pharmaceutical composition comprising an antibody an acceptable carrier.
10

The invention further provides for human DCR6 polypeptide, an antibody, or a composition for use in a method of treatment of the human or animal body, or in a method of diagnosis.

15 The invention provides for a ligandbody which comprises human DCR6 fused to an immunoglobulin constant region and a ligandbody wherein the immunoglobulin constant region is the Fc portion of human IgG1.

The invention provides for a ligandbody for use in a method of treatment of the
20 human or animal body, or in a method of diagnosis.

Another embodiment of the invention is a recombinant nucleic acid encoding DCR6 polypeptide comprising the amino acid sequence as set forth in Figure 2A-2B or Figure 3A-3B or a fragment thereof having DCR6- specific activity.
25

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth in Figure 2A-2B or Figure 3A-3B or a fragment thereof having at least 18 consecutive bases of the sequences set forth in Figure 2A-2B or Figure 3A-3B and sufficient to specifically hybridize with a nucleic acid having
30 the sequences as set forth in Figure 2A-2B or Figure 3A-3B in the presence of natural DCR6 cDNA.

The present invention also provides for antibodies to the DCR6 polypeptide described herein which are useful for detection of the polypeptide in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward this DCR6 polypeptide, any technique which provides for the
5 production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature* 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole
10 et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies for diagnostic or therapeutic use may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal
15 antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:7308-7312; Kozbor et al., 1983, *Immunology Today* 4:72-79; Olsson et al., 1982, *Meth. Enzymol.* 92:3-16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions
20 (Morrison et al., 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81:6851, Takeda et al., 1985, *Nature* 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of the DCR6 polypeptide described herein. For
25 the production of antibody, various host animals can be immunized by injection with the DCR6 polypeptide, or a fragment or derivative thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as
30 aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, polypeptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille

Calmette-Guerin) and Corynebacterium parvum.

- A molecular clone of an antibody to a selected DCR6 polypeptide epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g.,
- 5 Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.
- 10 The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be
- 15 generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a
- 20 combination thereof.

The invention further provides for a method of using a DCR6 polypeptide or fragment thereof as an antagonist of the activity of a bone morphogenic protein (BMP).

25 The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Cloning and Sequencing of "Virtual" Human DCR6

5

A. "Virtual" cloning

The Human Virtual Transcribed Sequence Database (Kazusa DNA Research Institute, <http://zearth.kazusa.or.jp/vts/intro.html>), is a database that contains
10 protein sequences that are predicted to be encoded by human genomic sequences. The Human Virtual Transcribed Sequence Project aims to provide candidate transcribed sequences from the available human genome sequencing data by using the gene detection method, GENSCAN (see *infra*) by Chris Burge (cburge@mit.edu). Therefore it is entirely *in silico* gene cloning.

15

Currently, the database is collecting human genome sequence data from Genbank gss, htg, new, pri1, pri2, entries and from the Web pages of Lawrence Berkeley
National Laboratory Human Genome Center, Whitehead Institute/MIT Genome Sequencing Project, The Sanger Centre, Washington University Genome
20 Sequencing Center, Genome Therapeutics Corporation, Japan Science and Technology Corporation, and Yale Center for Medical Informatics.

VTs has been developed by Nobuyuki Miyajima (miyajima@kazusa.or.jp, Kazusa DNA Research Institute) and Toshiyuki Saito (t_saito@nirs.go.jp,
25 National Institute of Radiological Sciences).

GENSCAN is a program designed to predict complete gene structures, including exons, introns, promoter and polyadenylation signals, in genomic sequences. It differs from the majority of existing gene finding algorithms in that it allows for
30 partial genes as well as complete genes and for the occurrence of multiple genes in a single sequence, on either or both DNA strands. The program is based on a probabilistic model of gene structure/compositional properties and does not

make use of protein sequence homology information. The text output of the program is a list of one or more (or possibly zero) predicted genes together with the corresponding peptide sequences. The graphical output (PostScript or gif) is a diagram of the locations of the predicted exons.

5

In an attempt to clone novel members of the DAN/Cerberus family, the Human Virtual Transcribed Sequence Database was searched by querying with the sequences of several different DAN/Cerberus family members, including the human DCR5 sequence as set forth in co-pending US Provisional Application
10 No. 60/097,296, filed August 20, 1998). A "virtual" predicted polypeptide sequence sharing homology to the human DCR5 query sequence was identified and the corresponding genomic DNA sequence was obtained from the NCBI database (<http://www.ncbi.nih.gov>; Entrez Search System, nucleotides, Accession #AC003098). This genomic DNA sequence, designated virtual Human
15 DAN/Cerberus related protein 6 (vts_hDCR6) was used to design oligonucleotide primers for use in a PCR-based homology cloning strategy to determine if the "virtual" sequence was in fact transcribed *in vivo*.

Vts_hDCR6 was identified as a predicted open reading frame (ORF) encoding a
20 polypeptide that shares sequence homology with the DAN/Cerberus protein family. The vts_hDCR6 genomic DNA sequence and the regions corresponding to the predicted open reading frame consisting of four exons is set forth in Figure 1A-1F. Because vts_hDCR6 is only a predicted ORF identified by a computer algorithm, it was necessary to (a) show that hDCR6 is expressed in human
25 tissues, (b) determine if the predicted ORF has the same sequence as any actual cDNA clone of hDCR6, and (c) demonstrate that it is a secreted polypeptide.

B. PCR-amplification and cloning of vts hDCR6 exons 1, 2, 3, and 4:

30 The predicted four exons comprising vts_hDCR6 that are set forth in Figure 1A-1F were each PCR-amplified independently using the following oligonucleotide primers:

Exon 1:**mts_DCR6.ex1 PCR5' (Sal I):**

CAG ATA GTC GAC GCC GCC ACC ATG GTG CTC CCA CTG GCC CTG TGT
5 CTC GTC TGC

mts_DCR6.ex1 PCR3' (Spe I):

CTC GAC TAG TGC TTT GGT CTC AAA GGG GTG GTG GGG AGG

10 Exon 2:**mts_DCR6.ex2 PCR5' (Spe I):**

AAA GCA CTA GTC GAG GAA CAG TCT TGC CTG GAG GTG

mts_DCR6.ex2 PCR3' (Eae):

15 CTC GGC CAC CTT GTT CCC TTC CCA GTG GTA CCA GCA GCT

Exon 3:**mts_DCR6.ex3 PCR5' (Eae):**

CAT GTG GCC GAG AAG TCC ACT GCC CAG GCT
20

mts_DCR6.ex3 PCR3' (Afl 3):

CTC GGA CAC GTA GCC CTT CAG GCA GTC GCT GGA GCC

Exon 4:**25 mts_DCR6.ex4 PCR5' (Afl 3):**

CAG TAC GTG TCC GAG TAC AGC TGC CGC GAG

mts_DCR6.ex4 PCR3' (Not I):

GTA GCG GCC GCC TAG TAG GCG TTC TCC AGC TCG GCC TG
30

Exons 1, 2, and 3 were PCR-amplified from human genomic DNA using the ExTaq DNA Polymerase PCR system (Panvera, Madison, WI, Cat. #TAKRR001C).

Exon 4 was amplified from human genomic DNA using the ExTaq DNA Polymerase PCR system in conjunction with PCR_x Enhancer System (Life Technologies, Inc., Rockville, MD, Cat. # 11495-017). Each PCR-amplified exon was subcloned into the pUC18 vector using the SureClone Ligation Kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden, Cat. #27-9300-01) and standard genetic engineering methodologies (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY). The sequence of each exon was verified using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA).

The complete ORF encoding vts_hDCR6 was then genetically engineered by piecing together the four individual exons into the expression vector pCS107 using standard techniques familiar to one of skill in the art. In order to facilitate reconstruction of the vts_hDCR6 ORF into this expression vector, it was necessary to introduce restriction sites between exons to allow for ligating the individual pieces in one unit. However, in each instance, the introduction of restriction sites resulted in silent mutations that did not alter the polypeptide sequence. The sites of exon boundaries are underlined in the sequence set forth in Figure 2A-2B. In addition to the silent mutations described *supra*, the second codon of vts_hDCR6 was changed from CAG to GTG to accommodate a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote efficient translational initiation.

Example 2: Northern blot analysis to evaluate the expression profile of hDCR6.

To determine whether vts_hDCR6 is expressed in human tissues, Multiple Tissue Northern blots (Clontech, Palo Alto, CA, Cat. # 7760-1, 7759-1, 7767-1, and 7765-1) were probed using standard Northern blot methodology with a ³²P-labeled nucleic acid fragment of vts_hDCR6 consisting of exons 1, 2, and 3. Exon

4 was omitted because its sequence is very GC-rich and as a result is prone to high background levels of non-specific hybridization. The results of the Northern analysis revealed low levels of hDCR6 mRNA expression in the adult kidney and very low levels of expression in heart muscle and colon. The size of the hDCR6 mRNA transcript was approximately 2.4kb.

Example 3: Cloning of hDCR6 by screening human kidney cDNA and a human kidney cDNA library:

- 10 Based on the results obtained in the Northern analysis, human kidney cDNA (Clontech, Palo Alto, CA, Cat. #7405-1) was used as a template in the following PCR-based gene cloning strategy. Using the 5' oligonucleotide primer used to amplify exon 1 of vts_hDCR6 (vts_DCR6.ex1 PCR5' (Sal I)) and the 3' oligonucleotide primer used to amplify exon 4 of vts_hDCR6 (vts_DCR6.ex4 PCR3' (Not I)) and human kidney cDNA as a template, a PCR reaction was
- 15 performed. Unexpectedly, the PCR reaction resulted in the amplification of an approximately 0.7kb DNA fragment, rather than the expected 1.2kb fragment predicted by the vts_hDCR6 ORF. Because the size of this fragment was smaller than that expected for vts_hDCR6, it was reasoned that the splicing of the hDCR6 mRNA differed from that of vts_hDCR6. To verify this, the PCR-derived DNA
- 20 fragment was directly sequenced by standard techniques. The sequence revealed that hDCR6 as expressed in kidney was comprised of exons 1 and 4 of vts_hDCR6 and not any sequence associated with exons 2 and 3.
- 25 To obtain a cDNA clone of hDCR6, a human kidney cDNA Rapid-Screen cDNA Library Panel (Origene Technologies, Inc., Rockville, MD, Cat. #LKD-1001) was screened by PCR using the same oligonucleotide primers (vts_DCR6.ex1 PCR5' (Sal I) and vts_DCR6.ex4 PCR3' (Not I)). A full length cDNA clone of hDCR6, comprising only exons 1 and 4 was thus obtained and sequence-verified. The
- 30 nucleic acid and deduced amino acid sequence of this hDCR6 clone is set forth in Figure 3A-3B. Using the computer program MacVector, it is predicted that the approximately first 20 amino acids encode a signal peptide sequence.

Example 4: Expression pattern of DCR6 in rat tissues.

As described *supra*, Northern analysis revealed that the expression of human DCR6 in adult human tissues is highly restricted to the heart, kidney, and colon (see Table 1).

5

TABLE 1

Tissue	relative level of expression of hDCR6
adrenal gland	undetectable
bladder (muscle only)	undetectable
bone marrow	undetectable
brain	undetectable
colon (mucosa lining)	low
colon (no mucosa) (muscle only)	undetectable
heart	low
heart (muscle only)	medium
kidney	high
liver	undetectable
lung	undetectable
lymph node	undetectable
ovary	undetectable
pancreas	undetectable
peripheral blood leukocytes	undetectable
placenta	undetectable
prostate	undetectable
prostate (muscle only)	undetectable
skeletal muscle	undetectable
skeletal (muscle only)	undetectable
small intestine	undetectable
small intestine (muscle only)	undetectable
spinal chord	undetectable
spleen	undetectable
stomach	undetectable
stomach (muscle only)	undetectable
testis	undetectable
thymus	undetectable
thyroid	undetectable
trachea	undetectable
uterus (no endometrium) (muscle only)	undetectable

Because these data do not yield any information as to which part of the tissue and which cell type(s) human DCR6 is expressed in, the expression of rat DCR6 was also examined in rat embryos at embryonic day 15 (E15) and in adult rat kidneys, using standard *in situ* hybridization techniques. Consecutive sections were

5 hybridized either to a sense or an anti-sense rat DCR6 probe and those tissues that hybridized to the anti-sense but not the sense probe were considered to be positive. By this criteria, rat DCR6 was found to be expressed throughout the choroid plexus (in the brain), in the dorsal surface of the tongue, in the pulmonary artery and aorta, the iliac artery, the lower intestine, and the

10 developing whisker follicles (follicles of vibrissa). There was also expression in the liver either in the lymphatic channels or in the portal veins. In the adult rat kidney, expression of rat DCR6 was restricted to the glomeruli. The association of DCR6 expression with vascular structures indicates that DCR6 may play an important role in the development and homeostasis of these structures. It is also

15 possible that in different diseases (e.g. kidney fibrosis) DCR6 may play an important role.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be

20 readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding human DCR6.
- 5 2. An isolated nucleic acid molecule as in claim 1 having a sequence selected from the group consisting of:
 - (a) the nucleotide sequence comprising the coding region of the human DCR6 as set forth in Figure 2A-2B;
 - (b) the nucleotide sequence comprising the coding region of the human DCR6 as set forth in Figure 3A-3B;
 - 10 (c) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) or (b) and which encodes DCR6; or
 - (d) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleic acid of (a), (b) or (c) and which encodes DCR6.
- 15 3. A vector which comprises a nucleic acid molecule of claim 1 or 2.
4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.
- 20 5. A vector according to claim 3 which is a plasmid.
- 25 6. Isolated human DCR6 polypeptide.
7. Isolated human DCR6 polypeptide, having the amino acid sequence as set forth in Figure 2A-2B.
- 30 8. Isolated human DCR6 polypeptide, having the amino acid sequence as set forth in Figure 3A-3B.

9. A host-vector system for the production of human DCR6 which comprises a vector of claim 3, in a host cell.
10. A host-vector system according to claim 9, wherein the host cell is a bacterial, yeast, insect or mammalian cell.
11. A method of producing human DCR6 which comprises growing cells of a host-vector system of claim 9, under conditions permitting production of the human DCR6, and recovering the human DCR6 so produced.
12. An antibody which specifically binds the human DCR6 of claim 6, 7, or 8.
13. An antibody according to claim 12, which is a monoclonal antibody.
14. A pharmaceutical composition comprising human DCR6 according to claim 6, 7, or 8, and an acceptable carrier.
15. A pharmaceutical composition comprising an antibody according to claim 12 and an acceptable carrier.
16. Human DCR6 according to claim 6, 7, or 8 for use in a method of treatment of the human or animal body, or in a method of diagnosis.
17. An antibody according to claim 12 for use in a method of treatment of the human or animal body, or in a method of diagnosis.
18. A composition according to claim 14 for use in a method of treatment of the human or animal body, or in a method of diagnosis.
19. A polypeptide produced by the method of claim 11.

20. A ligandbody which comprises human DCR6 fused to an immunoglobulin constant region.
21. The ligandbody of claim 20, wherein the immunoglobulin constant region is the Fc portion of human IgG1.
22. A ligandbody according to claim 20 or 21, for use in a method of treatment of the human or animal body, or in a method of diagnosis.

10

Figure 1A

10 20 30 40 50 60 70 80

TCATTGGCTG GCATGAAGCA GAGAGGGGCT TTAAAAAGGC GACCGTGTCT CGGCTGGAGA CCAGAGCCTG TGCTACTGGA

90 100 110 120 130 140 150 160

AGGTGGCGTG CCCTCCTCTG GCTGGTACCA TGCAGCTCCC ACTGGCCCTG TGTCTCGTCT GCCTGCTGGT ACACACAGCC

a a VTS_HDCR6 EXON 1 a

170 180 190 200 210 220 230 240

TTCCGTGTAG TGGAGGGCCA GGGGTGGCAG GCGTTCAAGA ATGATGCCAC GGAAATCATC CCCGAGCTCG GAGAGTACCC

a a a VTS_HDCR6 EXON 1 a a a

250 260 270 280 290 300 310 320

CGAGCCTCCA CCGGAGCTGG AGAACAACAA GACCATGAAC CGGGCGGAGA ACGGAGGGCG GCCTCCCCAC CACCCCTTTG

a a a VTS_HDCR6 EXON 1 a a a

330 340 350 360 370 380 390 400

AGACCAAAGG TATGGGGTGG AGGAGAGAAT TCTTAGTAAA AGATCCTGGG GAGGTTTTAG AAACCTTCTCT TTGGGAGGCT

>

410 420 430 440 450 460 470 480

TGGAAGACTG GGGTAGACCC AGTGAAGATT GCTGGCCTCT GCCAGCACTG GTCGAGGAAC AGTCTTGCTT GGAGGTGGGG

b VTS_HDCR6 EXON 2 b

490 500 510 520 530 540 550 560

GAAGAATGGC TCCTGGTGC AGCCTTCAAA TTCAGGTGCA GAGGCATGAG GCAACAGACG CTGGTGAGAG CCCAGGGCAG

b b b VTS_HDCR6 EXON 2 b b b

570 580 590 600 610 620 630 640

GGAGGACGCT GGGGTGGTGA GGGTATGGCA TCAGGGCATC AGAACAGGCT CAGGGGCTCA GAAAAGAAAA GGTTCCTAAAG

b b b VTS_HDCR6 EXON 2 b b b

650 660 670 680 690 700 710 720

AATCTCCTCC TGGGAATATA GGAGCCACGT CCAGCTGTG GTACCACTGG GAAGGGAACA AGGTAAGGGA GCCTCCCATC

b b VTS_HDCR6 EXON 2 b b b

730 740 750 760 770 780 790 800

CACAGAACAG CACCTGTGGG GCACCGGACA CTCTATGCTG GTGGTGGCTG TCCCCACCAC ACAGACCCAC ATCATGGAAT

810 820 830 840 850 860 870 880

CCCCAGGAGG TGAACCCCCA GCTCGAAGGG GAAGAAACAG GTTCCAGGCA CTCAGTAACT TGGTAGTGAG AAGAGCTGAG

890 900 910 920 930 940 950 960

GTGTGAACCT GGTTCATCC AACTGCAAGA TAGCCCTGGT GTGTGGGGGG GTGTGGGGGA CAGATCTCCA CAAAGCAGTG

2/10

Figure 1B

970 980 990 1000 1010 1020 1030 1040
* * * * *
GGGAGGAAGG CCAGAGAGGC ACCCCTGCAG TGTGCATGTC CCATGGCCTG CCCAGGGAGC TGGCACTTGA AGGAATGGGA
1050 1060 1070 1080 1090 1100 1110 1120
* * * * *
GTTTTCGGCA CAGTTTTCAGC CCCTGACATG GGTGCAGCTG AGTCCAGGCC CTGGAGGGGA GAGCAGCATC CTCTGTGCAG
1130 1140 1150 1160 1170 1180 1190 1200
* * * * *
GAGTAGGGAC ATCTGTCTTC AGCAGCCACC CCAGTCCCAA CCTTGCCCTCA TTCCAGGGGA GGGAGAAGGA AGAGGAACCC
1210 1220 1230 1240 1250 1260 1270 1280
* * * * *
TGGGTTCCTG GTCAGGCCTG CACAGAGAAG CCCAGGTGAC AGTGTGCATC TGGCTCTATA ATTGGCAGGA ATCCTGAGGC
1290 1300 1310 1320 1330 1340 1350 1360
* * * * *
CATGGGGCG TCTGAAATGA CACTTCAGAC TAAGAGCTTC CCTGTCTCTT GGCCATTATC CAGGTGGCAG AGAAGTCCAC
____VTS_HDCR6 E____>
1370 1380 1390 1400 1410 1420 1430 1440
* * * * *
TGCCAGGCT CCTGGACCCC AGCCCTCCCC GCCTCACAAC CTGTTGGGAC TATGGGGTGC TAAAAAGGC AACTGCATGG
____c____c____c____VTS_HDCR6 EXON 3____c____c____c____>
1450 1460 1470 1480 1490 1500 1510 1520
* * * * *
GAGGCCAGCC AGGACCCTCC GTCTTCAAAA TGGAGGACAA GGGCGCCTCC CCCCACAGCT CCCCTTCTAG GCAAGGTCAG
____c____c____c____VTS_HDCR6 EXON 3____c____c____c____>
1530 1540 1550 1560 1570 1580 1590 1600
* * * * *
CTGGGCTCCA GCGACTGCCT GAAGGGCTGT AAGGAACCCA AACACAAAAT GTCCACCTTG CTGGACTCCC ACGAGAGGCC
____VTS_HDCR6 EXON 3____>
1610 1620 1630 1640 1650 1660 1670 1680
* * * * *
ACAGCCCCTG AGGAAGCCAC ATGCTCAAAA CAAAGTCATG ATCTGCAGAG GAAGTGCTTG GCCTAGGGGC GCTATTCTCG
1690 1700 1710 1720 1730 1740 1750 1760
* * * * *
AAAAGCCGCA AAATGCCCCC TTCCCTGGGC AAATGCCCCC CTGACCACAC ACACATTCCA GCCCTGCAGA GGTGAGGATG
1770 1780 1790 1800 1810 1820 1830 1840
* * * * *
CAAACCAGCC CACAGACCAG AAAGCAGCCC CAGACGATGG CAGTGCCAC ATCTCCCTG CTGTGCTTGC TCTTCAGAGT
1850 1860 1870 1880 1890 1900 1910 1920
* * * * *
GGGGGTGGG GGTGGCCTTC TCTGTCCCT CTCTGGTTG GTCTTAAGAC TATTTTCAT TCTTCTTGT CACATTGGAA

3/10

Figure 1C

1930	1940	1950	1960	1970	1980	1990	2000
CTATCCCCAT	GAAACCTTTG	GGGGTGGACT	GGTACTCACA	CGACGACCAG	CTATTTAAAA	AGCTCCCACC	CATCTAAGTC
2010	2020	2030	2040	2050	2060	2070	2080
CACCATAGGA	GACATGGTCA	AGGTGTGTGC	AGGGGATCAG	GCCAGGCCTC	GGAGCCCAAT	CTCTGCCTGC	CCAGGGAGTA
2090	2100	2110	2120	2130	2140	2150	2160
TCACCATGAG	GCGCCCATTC	AGATAACACA	GAACAAGAAA	TGTGCCCAGC	AGAGAGCCAG	GTCAATGTTT	GTGGCAGCTG
2170	2180	2190	2200	2210	2220	2230	2240
AACCTGTAGG	TTTGGGTCA	GAGCTCAGGG	CCCCTATGGT	AGGAAAGTAA	CGACAGTAAA	AAGCAGCCCT	CAGCTCCATC
2250	2260	2270	2280	2290	2300	2310	2320
CCCCAGCCCA	GCCTCCCATG	GATGCTCGAA	CGCAGAGCCT	CCACTCTTGC	CGGAGCCAAA	AGGTGCTGGG	ACCCAGGGGA
2330	2340	2350	2360	2370	2380	2390	2400
AGTGGAGTCC	GGAGATGCAG	CCCAGCCTTT	TGGGCAAGTT	CTTTTCTCTG	GCTGGGCCCTC	AGTATTCTCA	TTGATAATGA
2410	2420	2430	2440	2450	2460	2470	2480
GGGGGTGGA	CACACTGCCT	TTGATTCCCT	TCAAGTCTAA	TGAATTCCTG	TCCTGATCAC	CTCCCTTCA	GTCCCTCGCC
2490	2500	2510	2520	2530	2540	2550	2560
TCCACAGCAG	CTGCCCTGAT	TTATTACCTT	CAATTAACCT	CTACTCCTTT	CTCCATCCCC	TGTCCACCCC	TCCCAAGTGG
2570	2580	2590	2600	2610	2620	2630	2640
CTGGAAAAGG	AATTGTTGGAG	AAGCCAGAGC	CAGGCAGAAG	GTGTGCTGAG	TACTTACCCT	GCCCAGGCCA	GGGACCCTGC
2650	2660	2670	2680	2690	2700	2710	2720
GGCACAAGTG	TGGCTTAAAT	CATAAGAAGA	CCCCAGAAGA	GAAATGATAA	TAATAATACA	TAACAGCCGA	CGCTTTCAGC
2730	2740	2750	2760	2770	2780	2790	2800
TATATGTGCC	AAATGGTATT	TTCTGCATTG	CGTGTGTAAT	GGATTAACTC	GCAATGCTTG	GGGCGGCCCA	TTTTGCAGAC
2810	2820	2830	2840	2850	2860	2870	2880
AGGAAGAAGA	GAGAGGTAA	GGAACCTGCC	CAAGATGACA	CCTGCAGTGA	GCGATGGAGC	CCTGGTGTTC	GAACCCAGC
2890	2900	2910	2920	2930	2940	2950	2960
AGTCATTTGG	CTCCGAGGGG	ACAGGGTGCG	CAGGAGAGCT	TTCCACCAGC	TCTAGAGCAT	CTGGGACCTT	CCTGCAATAG

Figure 1D

2970 2980 2990 3000 3010 3020 3030 3040
ATGTTTCAGGG GCAAAAGCCT CTGGAGACAG GCTTGGCAAA AGCAGGGCTG GGGTGGAGAG AGACGGGCGG GTCCAGGGCA
3050 3060 3070 3080 3090 3100 3110 3120
GGGGTGGCCA GCGGGGCGGC CACCCTCAG CGCGCTCTC TCCACAGACG TGTCCGAGTA CAGCTGCCGC GAGCTGCACT
_____d_____VTS_HDCR6 EXON 4_____>
3130 3140 3150 3160 3170 3180 3190 3200
TCACCCGCTA CGTGACCGAT GGGCCGTGCC GCAGCGCCAA GCCGGTCACC GAGCTGGTGT GCTCCGGCCA GTCCGGCCCC
_____d_____d_____d_____VTS_HDCR6 EXON 4_____d_____d_____d_____>
3210 3220 3230 3240 3250 3260 3270 3280
GCGCGCTGCG TGCCCAACGC CATCGGCCGC GCAAGTGGT GCGGACCTAG TGGGCCCCGAC TTCCGCTGCA TCCCGGACCG
_____d_____d_____d_____VTS_HDCR6 EXON 4_____d_____d_____d_____>
3290 3300 3310 3320 3330 3340 3350 3360
CTACCGCGCG CAGCGCGTGC AGCTGCTGTG TCCCGGTGGT GAGGCGCCGC GCGCGCGCAA GGTGCGCCTG GTGGCCTCGT
_____d_____d_____d_____VTS_HDCR6 EXON 4_____d_____d_____d_____>
3370 3380 3390 3400 3410 3420 3430 3440
GCAAGTGCAA GCGCCTCACC CGCTTCCACA ACCAGTCGGA GCTCAAGGAC TTCGGGACCG AGGCCGCTCG GCCCGAGAAG
_____d_____d_____d_____VTS_HDCR6 EXON 4_____d_____d_____d_____>
3450 3460 3470 3480 3490 3500 3510 3520
GGCCGGAAGC CGCGGCCCGG CGCCCGGAGC GCCAAAGCCA ACCAGGCCGA GCTGAGAGAAC GCCTACTAGA GCCCGCCCCG
_____d_____d_____VTS_HDCR6 EXON 4_____d_____d_____d_____>
3530 3540 3550 3560 3570 3580 3590 3600
GCCCCTCCCC ACCGGCGGGG GCCCCGGCCC TGAACCCGCG CCCACATTT CTGTCTCTCTG CGCGTGGTTT GATTGTTTAT
3610 3620 3630 3640 3650 3660 3670 3680
ATTTCATGT AAATGCCTGC AACCCAGGGC AGGGGGCTGA GACCTTCCAG GCCCTGAGGA ATCCCGGGCG CCGGCAAGGC
3690 3700 3710 3720 3730 3740 3750 3760
CCCCCTCAGC CCGCCAGCTG AGGGGTCCCA CGGGGCAGGG GAGGGAATTG AGAGTCACAG AACTGAGCC ACGCAGCCCC
3770 3780 3790 3800 3810 3820 3830 3840
GCCTCTGGGG CCGCTACCT TTGCTGGTCC CACTTCAGAG GAGGCAGAAA TGAAGCATT TTCACCGCCC TGGGGTTTAA
3850 3860 3870 3880 3890 3900 3910 3920
AGGGAGCGGT GTGGGAGTGG GAAAGTCCAG GGACTGTTA AGAAAGTTGG ATAAGATTCC CCCTTGACCC TCGCTGCCCA

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Figure 1E

3930 3940 3950 3960 3970 3980 3990 4000
TCAGAAAGCC TGAGGCGTGC CCAGAGCACA AGACTGGGGG CAACTGTAGA TGTGGTTTCT AGTCCTGGCT CTGCCACTAA
4010 4020 4030 4040 4050 4060 4070 4080
CTTGCTGTGT AACCTTGAAC TACACAATTC TCCTTCGGGA CCTCAATTTC CACTTTGTAA AATGAGGGTG GAGGTGGGAA
4090 4100 4110 4120 4130 4140 4150 4160
TAGGATCTCG AGGAGACTAT TGGCATATGA TTCCAAGGAC TCCAGTGCCT TTTGAATGGG CAGAGGTGAG AGAGAGAGAG
4170 4180 4190 4200 4210 4220 4230 4240
AGAAAGAGAG AGAATGAATG CAGTTGCATT GATTCACTGC CAAGGTCCT TCCAGAATTC AGAGTTGTGA TGCTCTCTTC
4250 4260 4270 4280 4290 4300 4310 4320
TGACAGCCAA AGATGAAAAA CAAACAGAAA AAAAAAGTA AAGAGTCTAT TTATGGCTGA CATATTTACG GCTGACAAAC
4330 4340 4350 4360 4370 4380 4390 4400
TCCTGGAAGA AGCTATGCTG CTTCCTCAGCC TGGCTTCCCC GGATGTTTGG CTACCTCCAC CCCTCCATCT CAAAGAAATA
4410 4420 4430 4440 4450 4460 4470 4480
ACATCATCCA TTGGGGTAGA AAAGGAGAGG GTCCGAGGGT GGTGGGAGGG ATAGAAATCA CATCCGCCCC AACTTCCCAA
4490 4500 4510 4520 4530 4540 4550 4560
AGAGCAGCAT CCTTCCCCCG ACCCATAGCC ATGTTTTTAA GTCACCTTCC GAAGAGAAGT GAAAGGTTCA AGGACACTGG
4570 4580 4590 4600 4610 4620 4630 4640
CCTTGCAGGC CCGAGGGAGC AGCCATCACA AACTCACAGA CCAGCACATC CCTTTTGAGA CACCGCCTTC TGCCACCAC
4650 4660 4670 4680 4690 4700 4710 4720
TCACGGACAC ATTTCTGCCT AGAAAACAGC TTCTTACTGC TCTTACATGT GATGGCATAT CTTACACTAA AAGAATATTA
4730 4740 4750 4760 4770 4780 4790 4800
TTGGGGGAAA AACTACAAGT GCTGTACATA TGCTGAGAAA CTGCAGAGCA TAATAGCTGC CACCCAAAAA TCTTTTGTAA
4810 4820 4830 4840 4850 4860 4870 4880
AATCATTTCC AGACAACCTC TTACTTTCTG TGTAGTTTTT AATGTTTAAA AAAAAAAGT TTAAACAGA AGCACATGAC
4890 4900 4910 4920 4930 4940 4950 4960
ATATGAAAGC CTGCAGGACT GGTGTTTTTT TTGGCAATTC TTCCACGTGG GACTTGTCCA CAAGAATGAA AGTAGTGGTT

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Figure 1F

4970	4980	4990	5000	5010	5020	5030	5040
*	*	*	*	*	*	*	*
TTTAAAGAGT	TAAGTTACAT	ATTTATTTTC	TCACCTAAGT	TATTTATGCA	AAAGTTTTC	TTGTAGAGAA	TGACAATGTT
5050	5060	5070	5080	5090	5100	5110	5120
*	*	*	*	*	*	*	*
AATATTGCTT	TATGAATTAA	CAGTCTGTTC	TTCCAGAGTC	CAGAGACATT	GTTAATAAAG	ACAATGAATC	ATGACCGAAA
5130	5140	5150	5160	5170	5180	5190	5200
*	*	*	*	*	*	*	*
GGATGTGGTC	TCATTTTGTC	AACCACACAT	GACGTCATTT	CTGTCAAAGT	TGACACCCCTT	CTCTTGGTCA	CTAGAGCTCC
5210	5220	5230	5240	5250	5260	5270	5280
*	*	*	*	*	*	*	*
AACCTTGGAC	ACACCTTTGA	CTGCTCTCTG	GTGGCCCTTG	TGGCAATTAT	GTCTTCCTTT	GAAAAGTCAT	GTTTATCCCT
5290	5300	5310	5320	5330	5340	5350	5360
*	*	*	*	*	*	*	*
TCCTTTCCAA	ACCCAGACCG	CATTTCTTCA	CCCAGGGCAT	GGTAATAACC	TCAGCCTTGT	ATCCTTTTAG	CAGCCTCCCC
5370	5380	5390	5400	5410	5420	5430	5440
*	*	*	*	*	*	*	*
TCCATGCTGG	CTTCCAAAAT	GCTGTCTCTCA	TTGTATCACT	CCCCTGCTCA	AAAGCCTTCC	ATAGCTCCCC	CTTGCCCAGG
5450	5460	5470	5480	5490	5500	5510	5520
*	*	*	*	*	*	*	*
ATCAAGTGCA	GTTTCCCTAT	CTGACATGGG	AGGCCTTCTC	TGCTTGACTC	CCACCTCCCA	CTCCACCAAG	CTTCCTACTG
5530	5540	5550	5560	5570	5580	5590	5600
*	*	*	*	*	*	*	*
ACTCCAAATG	GTCATGCAGA	TCCCTGCTTC	CTTAGTTTGC	CATCCACACT	TAGCACCCCC	AATAACTAAT	CCTCTTTCTT
5610	5620	5630	5640	5650	5660	5670	5680
*	*	*	*	*	*	*	*
TAGGATTCAC	ATTACTTGTC	ATCTCTTCCC	CTAACCTTCC	AGAGATGTTC	CAATCTCCCA	TGATCCCTCT	CTCCTCTGAG

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Figure 2A

```

      GTG      10      20      30      40      50      60
      TTT      *      *      *      *      *      *
ATG CAG CTC CCA CTG GCC CTG TGT CTC GTC TGC CTG CTG GTA CAC ACA GCC TTC CGT GTA GTG GAG
Met Gln Leu Pro Leu Ala Leu Cys Leu Val Cys Leu Leu Val His Thr Ala Phe Arg Val Val Glu>

      70      80      90      100      110      120      130
      *      *      *      *      *      *      *
GGC CAG GGG TGG CAG GCG TTC AAG AAT GAT GCC ACG GAA ATC ATC CCC GAG CTC GGA GAG TAC CCC
Gly Gln Gly Trp Gln Ala Phe Lys Asn Asp Ala Thr Glu Ile Ile Pro Glu Leu Gly Glu Tyr Pro>

      140      150      160      170      180      190
      *      *      *      *      *      *
GAG CCT CCA CCG GAG CTG GAG AAC AAC AAG ACC ATG AAC CCG GCG GAG AAC GGA GGG CCG CCT CCC
Glu Pro Pro Pro Glu Leu Glu Asn Asn Lys Thr Met Asn Arg Ala Glu Asn Gly Gly Arg Pro Pro>

200      210      220      A      230      240      250      260
      *      *      *      *      *      *      *
CAC CAC CCC TTT GAG ACC AAA GCA CTG GTC GAG GAA CAG TCT TGC CTG GAG GTG GGG GAA GAA TGG
His His Pro Phe Glu Thr Lys Ala Leu Val Glu Glu Gln Ser Cys Leu Glu Val Gly Glu Glu Trp>

      270      280      290      300      310      320      330
      *      *      *      *      *      *      *
CTC GCT GGT GCA GCC TTC AAA TTC AGG TGC AGA GGC ATG AGG CAA CAG ACG CTG GTG AGA GCC CAG
Leu Ala Gly Ala Ala Phe Lys Phe Arg Cys Arg Gly Met Arg Gln Gln Thr Leu Val Arg Ala Gln>

      340      350      360      370      380      390
      *      *      *      *      *      *
GGC AGG GAG GAC GCT GGG GTG GTG AGG GTA TGG CAT CAG GGC ATC AGA ACA GGC TCA GGG GCT CAG
Gly Arg Glu Asp Ala Gly Val Val Arg Val Trp His Gln Gly Ile Arg Thr Gly Ser Gly Ala Gln>

400      410      420      430      440      450      460
      *      *      *      *      *      *      *
AAA AGA AAA GGT TTC AAA GAA TCT CCT CCT GGG AAT ATA GGA GCC ACG TCC AGC TGC TGG TAC CAC
Lys Arg Lys Gly Phe Lys Glu Ser Pro Pro Gly Asn Ile Gly Ala Thr Ser Ser Cys Trp Tyr His>

      470      480      C      490      500      510      520
      *      *      *      *      *      *      *
TGG GAA GGG AAC AAG GTG GCA GAG AAG TCC ACT GCC CAG GCT CCT GGA CCC CAG CCC TCC CCG CCT
Trp Glu Gly Asn Lys Val Ala Glu Lys Ser Thr Ala Gln Ala Pro Gly Pro Gln Pro Ser Pro Pro>

530      540      550      560      570      580      590
      *      *      *      *      *      *      *
CAC AAC CTG TTG GGA CTA TGG GGT GCT AAA AAG GGC AAC TGC ATG GGA GGC CAG CCA GGA CCC TCC
His Asn Leu Leu Gly Leu Trp Gly Ala Lys Lys Gly Asn Cys Met Gly Gly Gln Pro Gly Pro Ser>

      600      610      620      630      640      650      660
      *      *      *      *      *      *      *
GTC TTC AAA ATG GAG GAC AAG GGC GCC TCC CCC CAC AGC TCC CCT TCT AGG CAA GGT CAG CTG GGC
Val Phe Lys Met Glu Asp Lys Gly Ala Ser Pro His Ser Ser Pro Ser Arg Gln Gly Gln Leu Gly>

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Figure 2B

```

      670      680      690      700      710      720
      *      *      *      *      *      *
TCC AGC GAC TGC CTG AAG GGC TAC GTG TCC GAG TAC AGC TGC CGC GAG CTG CAC TTC ACC CGC TAC
Ser Ser Asp Cys Leu Lys Gly Tyr Val Ser Glu Tyr Ser Cys Arg Glu Leu His Phe Thr Arg Tyr>

      730      740      750      760      770      780      790
      *      *      *      *      *      *      *
GTG ACC GAT GGG CCG TGC CGC AGC GCC AAG CCG GTC ACC GAG CTG GTG TGC TCC GGC CAG TGC GGC
Val Thr Asp Gly Pro Cys Arg Ser Ala Lys Pro Val Thr Glu Leu Val Cys Ser Gly Gln Cys Gly>

      800      810      820      830      840      850
      *      *      *      *      *      *
CCG GCG CGC CTG CTG CCC AAC GCC ATC GGC CGC GGC AAG TGG TGG CGA CCT AGT GGC CCC GAC TTC
Pro Ala Arg Leu Leu Pro Asn Ala Ile Gly Arg Gly Lys Trp Trp Arg Pro Ser Gly Pro Asp Phe>

      860      870      880      890      900      910      920
      *      *      *      *      *      *      *
CGC TGC ATC CCC GAC CGC TAC CGC GCG CAG CGC GTG CAG CTG CTG TGT CCC GGT GGT GAG GCG CCG
Arg Cys Ile Pro Asp Arg Tyr Arg Ala Gln Arg Val Gln Leu Leu Cys Pro Gly Gly Glu Ala Pro>

      930      940      950      960      970      980      990
      *      *      *      *      *      *      *
CGC GCG CGC AAG GTG CGC CTG GTG GCC TCG TGC AAG TGC AAG CGC CTC ACC CGC TTC CAC AAC CAG
Arg Ala Arg Lys Val Arg Leu Val Ala Ser Cys Lys Cys Lys Arg Leu Thr Arg Phe His Asn Gln>

      1000     1010     1020     1030     1040     1050
      *      *      *      *      *      *
TCG GAG CTC AAG GAC TTC GGG ACC GAG GCC GCT CGG CCG CAG AAG GGC CGG AAG CCG CGG CCC CGC
Ser Glu Leu Lys Asp Phe Gly Thr Glu Ala Ala Arg Pro Gln Lys Gly Arg Lys Pro Arg Pro Arg>

      1060     1070     1080     1090     1100
      *      *      *      *      *
GCC CGG AGC GCC AAA GCC AAC CAG GCC GAG CTG GAG AAC GCC TAC TAG
Ala Arg Ser Ala Lys Ala Asn Gln Ala Glu Leu Glu Asn Ala Tyr ***>

```

Figure 3A

```

      10      20      30      40      50      60
      *      *      *      *      *      *
ATG CAG CTC CCA CTG GCC CTG TGT CTC GTC TGC CTG CTG GTA CAC ACA GCC TTC CGT GTA GTG GAG
Met Gln Leu Pro Leu Ala Leu Cys Leu Val Cys Leu Leu Val His Thr Ala Phe Arg Val Val Glu>
                                   (10)                                (20)                22

      70      80      90      100      110      120      130
      *      *      *      *      *      *      *
GGC CAG GGG TGG CAG GCG TTC AAG AAT GAT GCC ACG GAA ATC ATC CCC GAG CTC GGA GAG TAC CCC
Gly Gln Gly Trp Gln Ala Phe Lys Asn Asp Ala Thr Glu Ile Ile Pro Glu Leu Gly Glu Tyr Pro>
                                   (30)                                (40)                44

      140      150      160      170      180      190
      *      *      *      *      *      *
GAG CCT CCA CCG GAG CTG GAG AAC AAC AAG ACC ATG AAC CGG GCG GAG AAC CGA GGG CGG CCT CCC
Glu Pro Pro Pro Glu Leu Glu Asn Asn Lys Thr Met Asn Arg Ala Glu Asn Gly Gly Arg Pro Pro>
                                   (50)                                (60)                66

      200      210      220      230      240      250      260
      *      *      *      *      *      *      *
CAC CAC CCC TTT GAG ACC AAA GAC GTG TCC GAG TAC AGC TGC CGC GAG CTG CAC TTC ACC CGC TAC
His His Pro Phe Glu Thr Lys Asp Val Ser Glu Tyr Ser Cys Arg Glu Leu His Phe Thr Arg Tyr>
                                   (70)                                (80)                88

      270      280      290      300      310      320      330
      *      *      *      *      *      *      *
GTG ACC GAT GGG CCG TGC CGC AGC GCC AAG CCG GTC ACC GAG CTG GTG TGC TCC GGC CAG TGC GGC
Val Thr Asp Gly Pro Cys Arg Ser Ala Lys Pro Val Thr Glu Leu Val Cys Ser Gly Gln Cys Gly>
                                   (90)                                (100)                110

      340      350      360      370      380      390
      *      *      *      *      *      *
CCG GCG CGC CTG CTG CCC AAC GCC ATC GGC CGC GGC AAG TGG TGG CGA CCT AGT GGG CCC GAC TTC
Pro Ala Arg Leu Leu Pro Asn Ala Ile Gly Arg Gly Lys Trp Trp Arg Pro Ser Gly Pro Asp Phe>
                                   (120)                                (130)                132

      400      410      420      430      440      450      460
      *      *      *      *      *      *      *
CGC TGC ATC CCC GAC CGC TAC CGC GCG CAG CGC GTG CAG CTG CTG TGT CCC GGT GGT GAG GCG CCG
Arg Cys Ile Pro Asp Arg Tyr Arg Ala Gln Arg Val Gln Leu Leu Cys Pro Gly Gly Glu Ala Pro>
                                   (140)                                (150)                154

      470      480      490      500      510      520
      *      *      *      *      *      *
CGC GCG CGC AAG GTG CGC CTG GTG GCC TCG TGC AAG TGC AAG CGC CTC ACC CGC TTC CAC AAC CAG
Arg Ala Arg Lys Val Arg Leu Val Ala Ser Cys Lys Cys Lys Arg Leu Thr Arg Phe His Asn Gln>
                                   (160)                                (170)                176

      530      540      550      560      570      580      590
      *      *      *      *      *      *      *
TCG GAG CTC AAG GAC TTC GGG ACC GAG GCC GCT CGG CCG CAG AAG GGC CGG AAG CCG CGG CCC CGC
Ser Glu Leu Lys Asp Phe Gly Thr Glu Ala Ala Arg Pro Gln Lys Gly Arg Lys Pro Arg Pro Arg>
                                   (180)                                (190)                198

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Figure 3B

600		610		620		630		640							
*		*		*		*		*							
GCC	CGG	AGC	GCC	AAA	GCC	AAC	CAG	GCC	GAG	CTG	GAG	AAC	GCC	TAC	TAG
Ala	Arg	Ser	Ala	Lys	Ala	Asn	Gln	Ala	Glu	Leu	Glu	Asn	Ala	Tyr	***>
(200)								(210)						213	

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(71) Applicant (for all designated States except US): **REGENERON PHARMACEUTICALS, INC.** [US/US]: 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/55193 A3

(54) Title: HUMAN DAN/CERBERUS RELATED PROTEIN 6 (DCR6)

(57) Abstract: DAN/Cerberus Related protein 6 (DCR6) polypeptides and related nucleic acids are provided. Included are natural (DCR6) homologs from several species and polypeptides comprising a (DCR6) domain having specific activity. The polypeptides may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are isolated hybridization probes and oligonucleotide primers capable of specifically hybridizing with the disclosed genes, specific binding agents and methods of making and using the subject compositions.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 00/05537

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N5/10 C12N15/62 C12N15/63 C07K14/475 C07K16/22
A61K38/18 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>BIRREN B. ET AL.: "Homo sapiens chromosome 17, clone HRPC905N1, complete sequence"</p> <p>EMBL DATABASE ENTRY AC003098; ACCESSION NO. AC003098, 14 November 1997 (1997-11-14), XP002146329 cited in the application</p>	1,2
E	<p>WO 00 32773 A (DARWIN DISCOVERY LTD.) 8 June 2000 (2000-06-08) SEQ ID NO:1; SEQ ID NO:2</p> <p style="text-align: center;">-/-</p>	1-6,8-22

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05537

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	<p>PEARCE J.J. ET AL.: "A Mouse Cerberus/Dan-Related Gene Family" DEVELOPMENTAL BIOLOGY, vol. 209, no. 1, 1 May 1999 (1999-05-01), pages 98-110, XP002146330 the whole document</p>	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0032773 A	08-06-2000	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)